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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

BERNARD MALFROY-CAMINE

Application No.: 08/973,576

Filed: December 5, 1997

For: TRANSVASCULAR AND INTRACELLULAR DELIVERY OF LIPIDIZED PROTEINS

Examiner: R. Schwadron

Group Art Unit: 1644

Declaration of Bernard Malfroy-Camine Under 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Dr. Bernard Malfroy-Camine, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I am currently the President and CEO of Eukarion, Inc., a biotech company that I co-founded in 1991 to develop proprietary therapeutic agents directed against

intracellular targets for the treatment of human disease. One area of Eukarion's research has focused on the modification of antibodies and other proteins to facilitate their passage across cell membranes and their interaction with specific intracellular targets. This research has formed the basis for the above-referenced patent application.

3. In 1976, I graduated from Ecole Polytechnique (Paris, France). In 1978, I graduated from the University of Paris VI (Paris, France) with a Master of Science degree in Pharmacology/Experimental Therapeutics. In 1982, I was awarded my doctorate degree in Neurobiology from the University of Paris VI.

4. Since obtaining my Ph.D. in 1982, I have worked for a number of biotech companies and research institutes, including Eukarion, Inc. (Bedford, MA), Genentech, Inc. (South San Francisco, CA), Alkermes, Inc. (Cambridge, MA), CNRS (National Center for Scientific Research, France), Scripps Clinic (San Diego, CA), INSERM (Paris, France), *etc.* In addition, while at these various biotech companies and research institutes, I have been actively involved in the development of a number of pharmaceuticals. For instance, since co-founding Eukarion, Inc. in 1991, I have played an instrumental role in the development of synthetic catalytic free radical scavengers that are useful as antioxidants in the treatment and prevention of various diseases (*see*, U.S. Patent Nos. 5,403,834, 5,696,109, 5,827,880, 5,834,509 and 6,046,188). Moreover, while at Genentech, Inc., I played an instrumental role in the development of enkephalinase, which is useful for treating peptide-mediated mucus secretion and bronchoconstriction in the airway consequent to various diseases, *e.g.*, asthma, chronic bronchitis, *etc.* (*see*, U.S. Patent Nos. 5,780,025, 5,403,585, 5,262,178 and 4,960,700). In addition, while at Alkermes, Inc., I played an instrumental role in the development of receptor mediated permeabilizers (RMPs), *i.e.*, peptides that increase the permeability of the blood-brain barrier to molecules such as therapeutic agents or diagnostic agents (*see*, U.S. Patent Nos. 5,686,416, 5,506,206, 5,268,164 and 5,112,596). Further, while at INSERM, I was a key member of the academic team that designed acetorphan (Tiorfan™), which has been approved for use in Europe. As such, I have had a great deal of experience in the development of

pharmaceuticals that are useful for treating a variety of diseases and, thus, I am familiar with the various considerations that go into the development of such compounds.

5. As illustrated above, I have been an inventor or co-inventor on over twelve patent applications and issued patents. In addition, I have authored or co-authored over 80 publications in *Neurobiology*, *Pharmacology*, *Molecular Biology* and *Biochemistry*. Attached hereto as Exhibit A is a true copy of my *curriculum vitae*.

6. I am the named inventor on the above-referenced patent application. I have read and am familiar with the contents of this patent application. In addition, I have read the Office Action, dated November 6, 2000, received in the present application. It is my understanding that the Examiner is concerned that claims 1-5, 7-12, 24 and 29-34 are only enabled for the claimed method or composition using or comprising the lipid glycyldioctadecylamide, which is shown in the example section of the specification. According to the Examiner, the claims encompass lipidized proteins containing a lipid with a hydrocarbon tail of greater than 12 carbons. However, the Examiner states that according to Horan *et al.* (U.S. Patent No. 5,665,328), it is unpredictable whether lipidized proteins that contain an added hydrocarbon tail of greater than 12 carbons will localize intracellularly or localize in the cell membrane. For the reasons set forth below, I believe that the specification, coupled with information known in the art at the time the present invention was made, provides ample guidance for one of skill in the art to make and use any lipidized proteins, with a hydrocarbon tail of at least 12 carbons.

7. Before addressing the Examiner's concerns, it may be helpful to clarify the description in Horan *et al.* relied upon by the Examiner. Horan *et al.* generally describes bio-affecting compounds having a hydrocarbon substituent for binding *to the cell membrane surface* (*see, e.g.*, col. 3, lines 25-42). By contrast, the present invention describes lipidized proteins with a lipid substituent having a hydrocarbon tail of at least 12 carbons that are

capable of transvascular transport, enhanced organ uptake *and intracellular localization*. Thus, the problem being solved by Horan *et al.* differs from that of the present invention.

8. Moreover, Horan *et al.* does not state that it is unpredictable whether lipidized proteins that contain an added hydrocarbon tail of greater than 12 carbons will localize intracellularly. Rather, Horan *et al.* was concerned with determining suitable hydrocarbon tail length for binding a bio-affecting compound with the hydrocarbon tail *to the cell membrane surface*. For example, Horan describes that the number of linear carbons in the hydrocarbon tails of the compounds is an important factor in achieving binding of the compounds to the cell membrane surface (*see,* col. 3, lines 52-56). Horan *et al.* further states the following:

> Experience with use of cyanine derivatives as diagnostic agents indicates that hydrocarbon tails of less than 3 carbons causes the cyanine to penetrate the plasma membrane and the nuclear membrane of cells resulting in staining of RNA and DNA. If carbon tails have a length greater than 3 carbons and less than 12 carbons, the compound no longer binds RNA and DNA but responds to membrane potential and enters the mitochondria... When the sum of the linear carbons in the hydrocarbon tail(s), is 23 or greater the lipophilicity of the molecule is increased such that it is retained in the plasma membrane and will not leak or transfer to other cells.... Thus, there may be a practical limitation on the length of the hydrocarbon tail(s) *depending on the chemical nature of the bio-affecting moiety to which it is to be bound*. [Emphasis added]. *See* col. 3, line 56 to col. 4, line 12.

As shown in the emphasized passage, the length of the hydrocarbon tails suitable for binding the bio-affecting compounds to cell membrane surface depends on the chemical nature of the bio-affecting moiety to which it is bound. Therefore, Horan *et al.* does not support the Examiner's statement that it is unpredictable whether any lipidized protein that contains an added hydrocarbon tail of greater than 12 carbons will localize intracellularly or localize in the cell membrane.

9. In fact, the present specification provides a working example that a lipidized protein with a hydrocarbon tail of greater than 12 carbons, namely glycyldiooctadecylamide, is capable of localizing intracellularly. For example, glycyldiooctadecylamide was linked to bovine IgG (*see,* Example 1 at pages 30-31). This lipidized protein was labeled with ^{14}C, and was administered intravenously to mice. It was shown that the lipidized proteins were taken up into various organs, such as the brain, liver, spleen and kidney. In another example, a monoclonal antibody which specifically binds to the Tat protein of HIV-1 was lipidized with glycyldiooctadecylamide (*see,* Example 2 at page 32-33). It was shown that when cells were pretreated with the lipidized anti-Tat antibody prior to addition of HIV-1 virus, the treated cells were almost completely protected from the cytotoxic effects of the HIV-1 virus. By contrast, cells that were treated with native anti-Tat antibody or that were untreated were not protected from the cytotoxic effect of the virus. These results indicate that lipidized proteins with a hydrocarbon tail of greater than 12 carbons can localize intracellularly.

10. The specification also provides ample guidance for making and using other lipidized proteins with a lipid substituent having a hydrocarbon tail of at least 12 carbons for intracellular localization. For example, any lipids such as lipoamines, lipopolyamines, and fatty acids can be attached by a covalent linkage to a carbohydrate side chain of a protein (*see,* page 15 lines 25-29 of the specification). As an illustration, lipoamines with varying lengths of hydrocarbon chains are described at pages 17-18 of the specification. Methods for attaching these lipid substituents to proteins are described in, *e.g.*, page 18, lines 30-32 and in Example 1 of the specification. The present specification also provides a variety of methods for testing whether a lipidized protein localizes intracellularly. For example, as described above, lipidized proteins can be radiolabeled, and their uptake by organs can be evaluated (*see, e.g.*, page 31 of the specification). In another example, a lipid substituent can be attached to an anti-Tat antibody, and its ability to protect cells from HIV-1 virus infection can be evaluated (*see, e.g.*, pages 32 and 33 of the specification). In view of this and other portions of the specification, either alone or coupled with information known in the art at the time the above-referenced

patent application was filed, it is my scientific opinion that one of skill in the art would have been *readily* able to make and use other lipidized proteins and to determine which lipidized proteins will localize intracellularly without undue experimentation.

11. In conclusion, there is no compelling objective basis to believe that lipidized proteins having a lipid substituent other than glycyldioctadecylamide will not localize intracellularly. In fact, it is my scientific opinion that given the working example and guidance provided in the specification, one of skill in the art would have been able to make and use numerous lipidized proteins having hydrocarbon tails of at least 12 carbons for intracellular localization without undue experimentation.

Bernard Malfroy-Camine

July 1, 2001

Bernard Malfroy-Camine, Ph.D. Date

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